Reference: *Biol. Bull.*, **136**: 327–346. (June, 1969)

THE ENVIRONMENTAL AND HORMONAL CONTROL OF GROWTH AND REPRODUCTION IN THE ADULT FEMALE STONE CRAB, *MENIPPE MERCENARIA* (SAY)[1]

T. S. CHEUNG

Institute of Marine Sciences, University of Miami 33149

The growth and reproductive biology of the adult female stone crab, *Menippe mercenaria*, an economically important species in Florida, have never been investigated in the laboratory. These morphogenetic processes are influenced both by hormones and by seasonal changes in the environment. This study seeks to survey the relation between these processes, their endocrine control, and the changing environmental conditions provided in nature. Special attention has been paid to the interrelationship between growth and reproduction. This aspect has been studied by Bauchau (1961), Cheung (1966) and Démeusy (1964, 1965a, 1965b and 1965c) in *Carcinus maenas*, a boreal species. The present study employs adult female specimens of *M. mercenaria* since they continue to grow after reaching sexual maturity, unlike *Callinectes* in which growth ceases at maturity (Truitt, 1939).

The relationship between growth and reproduction was defined by (1) observing the occurrence of molting and spawning in a sample of the wild population; and (2) removing the eyestalks at different stages of the molting cycles and during intermolt periods occurring at different times of the year, because the eyestalks contained endocrine factors regulating these processes. Results of this study also yielded information on the life history of this species.

Materials and Methods

Adult stone crabs were collected in baited traps (opening 3 × 5 inches) which were set in Biscayne Bay, approximately one mile from the Institute of Marine Science, University of Miami. Only crabs above 45 mm in carapace width (CW) were used since mature ones had not been found below this size. In the laboratory, they were maintained in circulating sea water in glass tanks with slate bottom, each measuring 1 × 2 feet in area, or in wooden tanks, each with about the same volume as the glass aquaria. The crabs were isolated from each other in order to avoid loss through cannibalism. Crabs held in glass aquaria received normal room daylight supplemented by fluorescent lamps; those in the wooden tanks were exposed to normal room daylight without supplementation. The circulating water in the laboratory was pumped almost directly from the Bay, so that its fluctuations in salinity and temperature were found to follow closely the same patterns as those in nature. Experimenting under controlled systems of

[1] Contribution No. 1029 from the Institute of Marine Sciences, University of Miami. Work largely supported by Aldcomp Corporation.

constant salinity or temperature had been considered, but was rejected here in preference to the circulating sea water, since the former method could not yield useful information for the study of life history.

All the crabs were fed daily with approximately 4 grams of shrimp meat; the tanks and compartments were cleaned regularly. Each tank was examined daily to record molting, spawning, or death in the normal, as well as experimental, animals.

Eyestalks were removed after the method of Cheung (1964) adopted from Bliss (1953). This included anesthetizing the crabs by cooling, and carefully cauterizing. This technique, when performed rapidly, almost eliminated post-operative mortality.

The data describing the natural environment were based on water samples

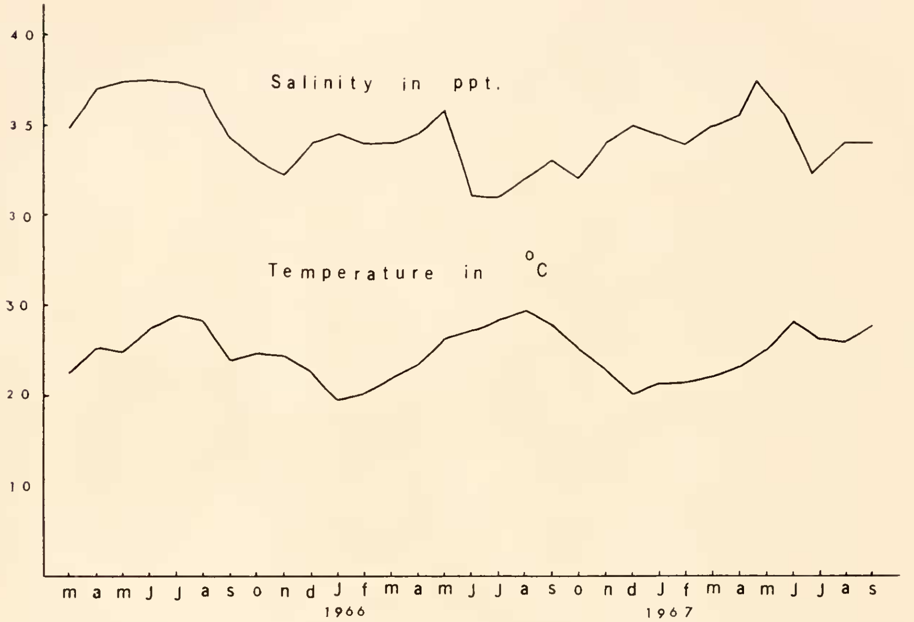

Figure 1. Salinity and temperature curves over period of study. These were monthly averages.

collected under the pier of the Institute. Similar data on salinities and temperatures were recorded daily from the laboratory tanks. Figure 1 gives the salinities and temperatures of the circulating water during the study period, which were found to be close to the figures obtained from water samples under the pier.

Results

Environmental factors

Since the same food was given daily to every crab, difference due to this factor was ruled out. No significant correlation could be found in spawning or molting with the lunar phase. The data may not be adequate to demonstrate this, or possibly, during prolonged exposure to laboratory conditions, the animals may have lost their rhythm. Therefore, this work has neither proved nor disproved the existence of a lunar periodicity.

The average monthly temperature ranged from 19.5° to 29° C during the study, with similar seasonal patterns in both years. As the spawning season had a more definite pattern, it is natural to assume that this would be related to temperature changes. Further, as changes in light intensity and photoperiod are related to those of temperature, the effects of light and temperature were distinguished whenever possible.

The average local salinity over the two-year span of this study varied between 29 and 38‰. Further, the seasonal changes in salinity during the two years were of an entirely different pattern, so that this factor has no apparent correlation with seasonal variations in spawning frequencies, which exhibited a similar pattern during the two years. The effects of salinity on molting, which displayed no regular seasonal pattern, are suggested in this crab (Noe, 1967) as well as certain other crabs, when the works of Bliss and Boyer (1964) and De Leersnyder (1967) are considered.

The best studies on environmental effects in this work are thus limited to temperature and light.

Table I

Data on molting and spawning of one female crab, which spawned the greatest number of times (13) yet known between two ecdyses

Molting: December 1, 1965; December 4, 1966.
Spawning: February 12, 1966; March 14, 1966; April 8, 1966; May 2, 1966; May 20, 1966; June 7, 1966; June 27, 1966; July 13, 1966; July 31, 1966; August 16, 1966; Sept, 2, 1966; Sept. 20, 1966; Oct. 4, 1966.

Endocrine control

The eyestalks are of particular interest not only because they contain the X-organs generally recognized to be responsible for the production of both molt-inhibiting (MIH) and ovarian-inhibiting (OIH) hormones (Carlisle and Knowles, 1959; Passano, 1960a) but because it is through them that visual stimuli are received. Thus they act as an "interface" between many external factors that may affect the MIH as well as OIH. The destalking experiments that follow are an attempt to study some relationships between the factors of external and internal environments.

Molting stages

While destalking of intermolt decapods has been found to accelerate molting, no acceleration due to the operation occurs if the animal is destalked during the premolt stage (Drach, 1944). There is little doubt that this generalization applies to all decapods. No results of destalking postmolt crabs have been published, probably because they are not easy to collect in sufficient numbers for experiments. During this study, however, a small number of crabs molted in the laboratory. These were destalked, with results to be described below.

Ecological results

Seasonal occurrence Between April, 1965 and February, 1967, 27 adult female crabs were maintained in the laboratory. Spawning and molting records in this group of animals were kept but not published here. They show that spawning was much more frequent than molting. Within a single intermolt period several spawnings could occur (Table I). Records of spawning and molting for the same animals during the experimental period are given in Figure 2, showing that they spawned 126 times, but molted only 29 times. There was thús an average of 4.5 spawnings within one single molt cycle in the laboratory. Further, the histogram representing spawning has its peak in the months of August and September. In October, spawning declined sharply. Spawning was at its lowest

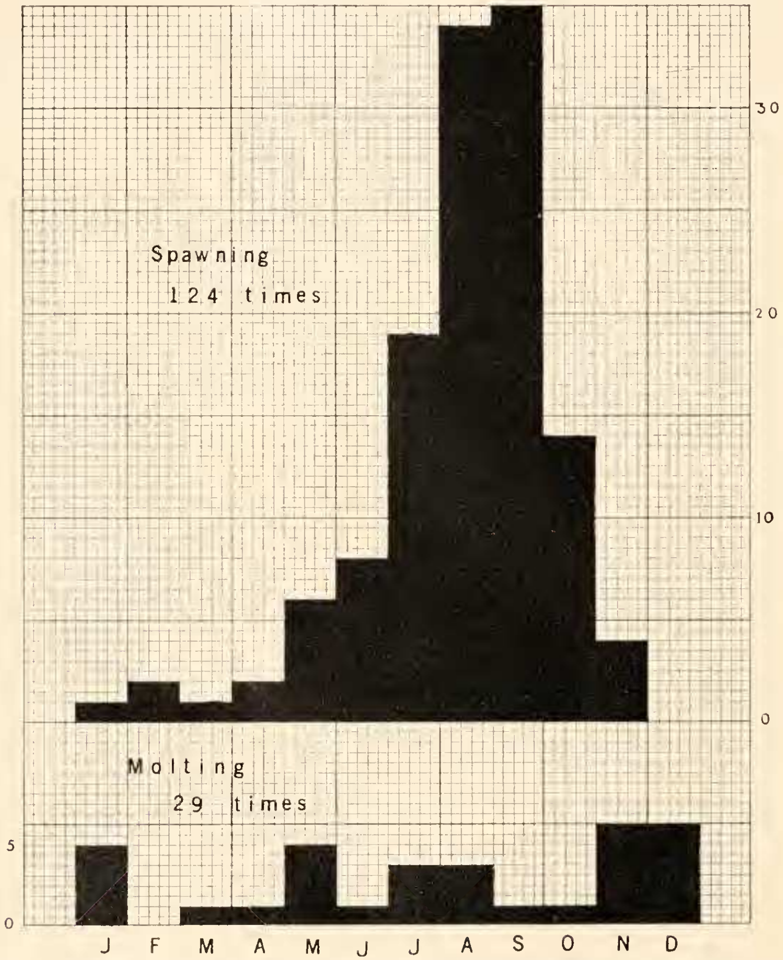

FIGURE 2. Histograms summing up records of spawning and molting for a group of stone crabs reared in the laboratory over a year under conditions described in the text.

Table II

All the female crabs listed in this Table spawned more than twice during the period of study. The position of "x" for each crab indicates the month in which the shortest inter-spawning period of the particular crab occurred. The total number of shortest inter-spawning periods in each month was derived by adding up the number of "x"s occurring in the month. Whenever the "x" lay between two months, one-half of it is credited to each month.

Crab no.	Jan.	Feb.	Mar.	Apr.	May	Jun.		Jul.		Aug.		Sep.		Oct.	Nov.	Dec.
1						x		x		x						
2							x									
4											x					
5b							x				x					
6													x			
7b													x			
8a											x					
10											x	x				
11											x					
12										x						
13								x				x				
14a												x				
15									x							
16									x							
18												x				
20										x						
21										x						
23									x							
28										x						
29											x					
30					x											
Total					1	2		4.5		9.5		8		1		

ebb in winter, in the months of November through March. This pattern is in good agreement with the results of sampling (Noe, 1967) in the same locality where these experimental animals were caught, during a period that overlapped the present experiments.

Of the 29 ecdyses of the above animals, 14 occurred between November 1 and January 31, in the same period when spawning least occurred. The other 15, however, were scattered irregularly over 7 months in the rest of the year. The molting season is therefore not as clearly defined as that of spawning. Because of the fact that temperature, salinity and photoperiodic conditions in the laboratory were similar to those in nature, it is reasonable to expect that the molting and spawning seasons of the laboratory population should agree with the field data.

Intervals between molting and spawning Ovarian development and molting are antagonistic processes in this species (Noe, 1967) as they are in *Carcinus maenas* (Bauchau, 1961; Cheung, 1966; Démeusy, 1963). Thus the length of the period between molting and spawning may indicate the rate of change in the physiological conditions of the animals; the particular seasons at which they occur may also reflect influence of seasonal factors.

Sixteen crabs were available for this study. The results are shown in Figure 3. All the spawnings took place at the C4 stage of the integument.

It is noteworthy that all the spawnings, with one exception, occurred in the

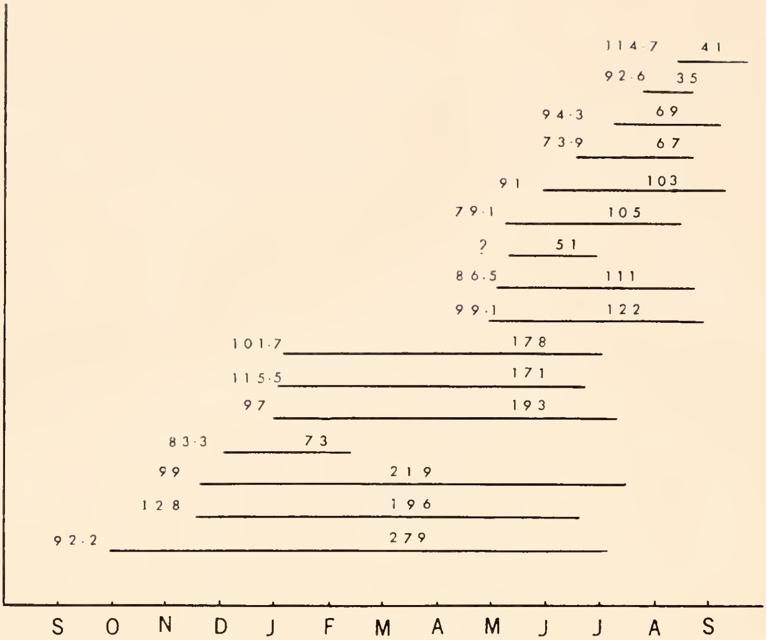

FIGURE 3. Observed periods from molting to spawning in 16 crabs reared in the laboratory. The number on the left side of each line represents the carapace width of a crab in millimeters. The number above each line indicates the number of days from molting to spawning.

summer months irrespective of the month in which the previous molt had taken place. This suggests that ovarian development depends on seasons and is independent of the length of time since the last molting occurred. The interval between molting and spawning bears no correlation with the size of the animals.

Since several spawnings could take place within one molt cycle, the comparative lengths of inter-spawning periods in any single animal reveal different rates of ovarian development at different times of the year.

Twenty-one crabs spawned more than twice within the same molt cycle. The distribution of the shortest inter-spawning intervals in time is illustrated in Table II. When the interval includes parts of two successive months, half of the period is considered to be in each of the two months. A single shortest inter-spawning period occurred in May, 2 in June, 4.5 in July, 9.5 in August, 8 in September and 1 in October. In the rest of the year there was no shortest inter-spawning period.

These results suggest that ovarian growth is most accelerated during the warmest month (average temperature 29° C) but not necessarily the month with longest day-length or highest light intensity. Any effect of light is probably restricted to the initial phase of ovarian development.

Data on 15 crabs were available for this study (Figs. 4). With one exception, the last spawnings were confined to autumn. The subsequent moltings, however, had a wide scatter over all seasons, ranging from late October to middle August of the next year, although the majority took place in winter. The intervals between the last spawnings and the subsequent moltings bear no correlation with the size of the crabs.

Only three adult female crabs molted twice during the period of study. One required 6 months, while the other two, 1 year. This information suggests that intermolt periods for the adult stone crabs are long.

Relationship between spawning and molting In Figure 2, the difference between the distribution of spawning and ecdysis is noteworthy. The histogram of spawning shows a normal distribution with a peak in August or September, suggesting that summer is the most favorable season. Moltings, on the other hand, show no definite peak, although about half of them occurred in the winter months. If winter were favorable for growth, as summer appears to be for spawning, the data should appear as a normal distribution curve with a peak in winter. But this relationship did not occur even though molting and ovarian development are antagonistic processes. There was no inverse correlation between the histograms. Further, from Figures 3 and 4, molting did not appear to be as dependent on season as spawning. Possibly it was affected by salinity changes which appeared rather irregularly in this area.

Conclusions from ecological observations Ovarian development of the crab was closely correlated with local water temperature. Optimal development was

TABLE III

Destalking experiment on adult female stone crabs kept in wooden tanks, August, 1966

Crab no.	CW (mm)	Date destalked	Date molted	Date spawned
4	54	Aug. 31, 1966	Sep. 19, 1966	Oct. 11, 1966
5	53	Aug. 31, 1966	Sep. 25, 1966	Oct. 18, 1966
6	71	Aug. 31, 1966	Oct. 12, 1966	—
7	92	Aug. 31, 1966	Oct. 1, 1966	—
8	72	Aug. 31, 1966	Sep. 23, 1966	Oct. 18, 1966 Jan. 3, 1967 Feb. 28, 1967
9	73	Aug. 30, 1966	Oct. 8, 1966	—
10	70	Sep. 1, 1966	Sep. 29, 1966	—
12	90	Aug. 31, 1966	Oct. 11, 1966	—
13	84	Aug. 31, 1966	Oct. 11, 1966	—
11	105	Aug. 31, 1966	Oct. 11, 1966	—
F9	87	—	—	—
F10	80	—	—	—
F11	72	—	—	—
F12	74	—	—	—
F13	76	—	Oct. 27, 1966	—
F14	95	—	—	—
F15	78	—	—	—
F16	45	—	—	—
F17	94	—	—	—
F18	97	—	—	—
F19	75	—	—	—
F20	64	—	—	—
F21	102	—	—	—

TABLE IV

Destalking experiment on adult female stone crabs kept in wooden tanks, November, 1966

Crab no.	CW (mm)	Date destalked	Date molted	Date spawned
F1	67	Nov. 2, 1966	Dec. 8, 1966	—
F2	94	Nov. 2, 1966	—	—
F3	95	Nov. 2, 1966	Jan. 1, 1967	—
F4	71	Nov. 2, 1966	Nov. 28, 1966	Jan. 18, 1967
F5	88	Nov. 2, 1966	Dec. 27, 1966	—
F6	55	Nov. 2, 1966	Dec. 10, 1966	—
F7	85	Nov. 2, 1966	Nov. 28, 1966	—
F8	77	Nov. 2, 1966	Dec. 27, 1966	—
F9	87	Nov. 2, 1966	Dec. 16, 1966	Feb. 20, 1967 Apr. 11, 1967 May 20, 1967
F10	80	Nov. 2, 1966	Dec. 19, 1966	Feb. 18, 1967 Apr. 9, 1967
F11	72	Nov. 3, 1966	Dec. 25, 1966	Mar. 28, 1967
F12	74	Nov. 3, 1966	Dec. 5, 1966	—
F14	95	Nov. 3, 1966	Dec. 12, 1966	Feb. 24, 1967
F15	78	—	—	—
F16	45	—	—	—
F17	94	—	—	—
F18	97	—	—	—
F19	75	—	—	—
F20	64	—	—	—
F21	102	—	—	—
F22	87	—	—	—
F23	82	—	—	—
F24	88	—	—	—
F25	114	—	—	—
F26	59	—	—	—
F27	94	—	—	—
F28	70	—	—	—
F29	67	—	—	—

seen at approximately 28° C. Longest day-length and highest light intensity do not correspond with fastest maturation of eggs. The age (as reflected by size) of the crab has no relation to the length of the developmental period of the eggs. Further, the spawning season is not affected by the time when the previous molting occurred. At the lowest ebb of spawning in winter, molting occurred in abundance (an exception to this was in February, the coldest month which is apparently unfavorable for either molting or spawning). These results suggest that growth was inhibited by reproductive activities but not *vice versa*. To clarify factors controlling growth and reproduction the following experiments were performed.

Seasonal changes in the morphogenetic effects of the eye-stalks at intermolt stage

MIH as well as OIH are produced by the eyestalk X-organs. Removal of the eyestalks has been found to cause either precocious molting or ovarian development in various species (Passano, 1953; Brown and Jones, 1949; Démeusy, 1962).

TABLE V

Destalking experiment on adult female stone crabs kept in wooden tanks, March, 1967

Crab no.	CW	Date destalked	Date molted	Date spawned	Dead
F17	94	Mar. 24, 1967	May 14, 1967	—	
F19	75	Mar. 24, 1967	Apr. 26, 1967	—	
F21	102	Mar. 24, 1967	—	—	Apr. 5, 1967
F23	82	Mar. 24, 1967	May 6, 1967	—	
F24	88	Mar. 24, 1967	—	Apr. 25, 1967	
F26	59	Mar. 24, 1967	Apr. 21, 1967	—	
F29	67	Mar. 24, 1967	Apr. 30, 1967	—	
F15	78	—	Jun. 7, 1967	—	
F16	45	—	Apr. 12, 1967	—	
F18	97	—	—	—	
F20	64	—	Jun. 10, 1967	—	
F22	87	—	May 25, 1967	—	
F25	114	—	—	May 4, 1967	
F27	94	—	—	—	
F28	70	—	May 22, 1967	—	

TABLE VI

Destalking experiment on adult female stone crabs kept in glass tanks, February, 1967

Crab no.	CW	Date destalked	Date molted	Date spawned
5	128	Feb. 7, 1967	Mar. 5, 1967	—
6	114.7	Feb. 7, 1967	—	Mar. 14, 1967
				Apr. 13, 1967
				May 16, 1967
10	86.5	Feb. 7, 1967	Mar. 30, 1967	Apr. 30, 1967
				Jun. 5, 1967
11	101.7	Feb. 7, 1966	—	Mar. 14, 1967
14	94.3	Feb. 7, 1967	—	Mar. 11, 1967
15	97	Feb. 7, 1967	Apr. 1, 1967	—
23	115.5	Feb. 7, 1967	—	Mar. 14, 1967
C	74	Feb. 7, 1967	Mar. 20, 1967	Apr. 27, 1967
				Jun. 20, 1967
B		Feb. 7, 1967	Mar. 30, 1967	—
4	91	—	—	Apr. 30, 1967
				May 20, 1967
				Jun. 5, 1967
G	81	—	—	May 12, 1967
				May 26, 1967
				Jun. 5, 1967
7	99.1	—	—	Apr. 28, 1967
29	92.6	—	—	Apr. 21, 1967
20	79.1	—	—	Jun. 18, 1967
H	73	—	—	—
13	92.2	—	—	May 21, 1967
1	98.1	—	—	—
21	99	—	—	May 18, 1967
				Jun. 1, 1967
34	91	—	May 12, 1967	—

These different responses have not been explained. Weitzman (1964) discovered that destalking specimens of *Gecarcinus lateralis* accelerated ovarian development in spring when such development normally occurs, but accelerated molting in the fall, when growth became dominant. This work indicated possible environmental influence on molting and maturation.

The following experiments on destalking stone crabs were designed to determine whether the activity of morphogenetic hormones varied with the change in seasons.

August to October, 1966 (Table III) All the crabs employed in this experiment were berried, and at the C4 stage (Passano, 1960a). The results show that destalking intermolt adult female crabs at this time invariably accelerated the subsequent molt.

November, 1966 to January, 1967 (Table IV) Both the destalked and control groups included control crabs from the last experiment as well as newly collected

TABLE VII

Destalking experiment on adult female stone crabs kept in glass tanks, April, 1967

Crab no.	CW	Date destalked	Date molted	Date spawned
4	91	Apr. 18, 1967	—	Apr. 20, 1967 May 20, 1967 Jun. 5, 1967
G	81	Apr. 18, 1967	—	May 2, 1967
1	98.1	Apr. 18, 1967	—	—
21	99	Apr. 18, 1967	—	May 18, 1967 Jun. 1, 1967
34	91	Apr. 18, 1967	May 12, 1967	—
7	99.1	—	—	Apr. 28, 1967
29	92.6	—	—	Apr. 21, 1967 May 16, 1967
20	79.1	—	—	—
H	73	—	—	—
13	92.2	—	—	May 21, 1967

crabs. In the beginning of this experiment no crab was berried. Destalking at this time also caused an acceleration of molting. The reaction of both the old and the newly collected crabs was the same.

February to June, 1967 During this period, 3 experiments were performed. The first one (Table V) employed all the control crabs from the previous experiment. Fifteen crabs were available, of which 8 were held as controls, while the other 7 were destalked. Of the 7 destalked crabs, 5 responded by molting; one by spawning; and one died without yielding any results. Five of the control crabs molted, 1 spawned while 2 neither molted nor spawned during the period of observation.

During the second experiment (Table VI) the light intensity was increased while other environmental conditions were not different from those of the first experiment. The control group contained 10 crabs, the destalked group 9. During the period of observation, 5 destalked crabs molted, 4 spawned. Of these numbers 6 and 10 spawned more than once. One of the control crabs molted, 8 spawned, while 2 neither molted nor spawned. Again, some of them spawned more than once.

These 2 experiments yielded results different from those of the previous ones in that the crabs responded to destalking by spawning as well as molting.

The third experiment (Table VII) was also conducted with elevated levels of illumination identical with that in Table VI. There were 5 control and 5 destalked crabs. During the observation period, 3 of the destalked animals spawned (2 more than once), 1 molted and 1 yielded no results. Of the controls, 3 spawned, a reminder that this was the spawning season.

Table VIII

Destalking experiment on adult female stone crabs, September, 1967.
(Crabs kept in wooden tanks, above line; Crabs kept in glass tanks, below line)

Crab no.	CW	Date destalked	Date molted	Date spawned
B1	92	Sep. 20, 1967	—	Sep. 29, 1967
B2	96	Sep. 20, 1967	—	Oct. 4, 1967
B3	117	Sep. 20, 1967	—	Oct. 11, 1967
				Oct. 24, 1967
B4	123	Sep. 20, 1967	—	Oct. 11, 1967
B5	96	Sep. 22, 1967	—	Oct. 4, 1967
				Oct. 29, 1967
F15	88	Sep. 21, 1967	—	Oct. 13, 1967
F27	94	Sep. 21, 1967	—	Oct. 10, 1967
F28	82	Sep. 21, 1967	—	Sep. 29, 1967
				Oct. 19, 1967
F24	98	Sep. 21, 1967	—	—
47	94	Sep. 5, 1967	—	Sep. 25, 1967
13	92.2	Sep. 5, 1967	—	Sep. 14, 1967
				Sep. 26, 1967
				Oct. 26, 1967
20	91	Sep. 5, 1967	—	Oct. 11, 1967
45	76	Sep. 5, 1967	—	Sep. 17, 1967
44	94	Sep. 5, 1967	—	Sep. 24, 1967
				Oct. 16, 1967
				Oct. 19, 1967
7	99.1	Sep. 5, 1967	—	Sep. 18, 1967
				Oct. 6, 1967
29	92.6	Sep. 5, 1967	—	Sep. 15, 1967
				Oct. 14, 1967
H	73	Sep. 5, 1967	—	Sep. 15, 1967
46	104	Sep. 5, 1967	—	Sep. 15, 1967

September 20th to November 5th, 1967 Eighteen crabs in two groups with different levels of illumination were destalked and observed. All operated crabs spawned (Table VIII), irrespective of the levels of illumination.

Conclusions to destalking experiments on intermolt crabs Two important points deserve attention: These results resemble those of Weitzman (1964) on *Gecarcinus* in that removal of the eyestalks may cause either spawning or molting in the adult female, depending on whatever process was dominant in the seasons when destalking was performed. (In this work, I am using spawning instead of ovarian growth as a criterion of reproduction.) In *Menippe,* between the time when molting was the dominant developmental process and when spawning replaced it, there was a

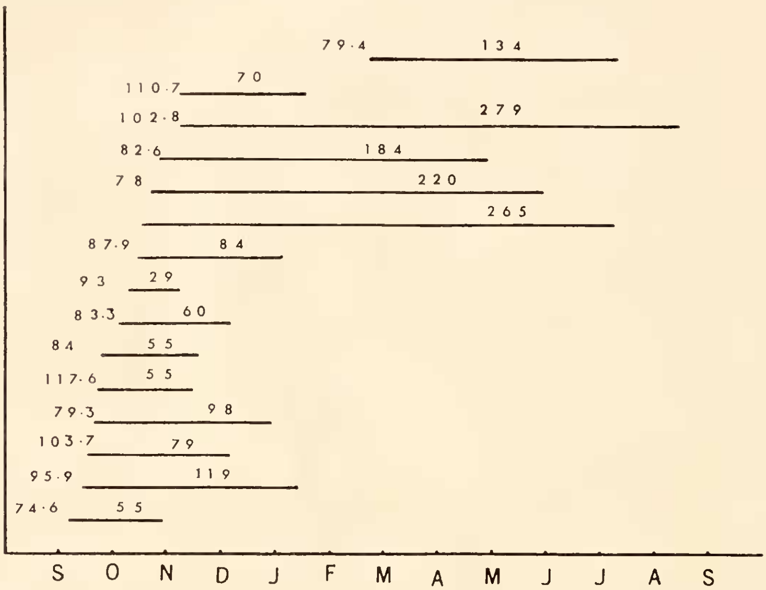

FIGURE 4. Observed periods from the last spawning to molting in 15 crabs reared in the laboratory. The number on the left side of each line represents the carapace width of a crab in millimeters. The number above each line indicates the number of days from spawning to molting.

transitional period (between January and September 1967) during which destalking might produce either molting or spawning, with the eventual complete dominance of spawning. The transition back to complete dominance of molting had not been followed, but would probably be more abrupt, judging by the fact that the limit of the spawning period is more sharply governed by the seasonal change (Figs. 3 and 4).

Secondly, the response to destalking in the autumns of 1966 and 1967 was entirely different. During the first autumn, the response was molting whereas during the second, it was spawning. This together with the inconsistancy of the molting season possibly due to the irregular changes in salinity, may be considered to reflect its ill-defined nature.

The environmental factors that brought about the seasonal difference in destalking effects thus appeared to be temperature and possibly light, but the contrasting results in the autumns of the two years were probably due to salinity. Unfortunately, due to the small number of experimental animals in (c) which included experiments under different lighting conditions, it was not possible to conclude the role light played. The positive effect on spawning of warm temperature occurring locally seems evident. The effect of lowering salinity on molting has been discussed; the effect of temperature will be described in the next section.

Effect of destalking on molting under different temperature conditions.

In the previous section, it was shown that spawning is restricted to the warmer months of the year. This section is only concerned with the effect of temperature on molting. If environmental factors in one season are more favorable than another it might be expected that destalked crabs will respond to the operation faster in the more favorable season. Results on three of the above experiments that provide data bearing on this aspect of the problem are compared in Table IX, in which time between destalking and molting is recorded. In the group of crabs destalked at the end of August, the average number of days before molting was

TABLE IX

The effect of destalking on molting under different temperature ranges

Crab no.	CW	Days between operation & molting	
4	54	19	
5	53	25	Average temperature: 28° C
6	71	42	Average CW: 72.0 mm
7	92	32	Average days: 32.4
8	72	23	Destalking month: August–September, 1966
9	73	49	Or: average CW: 78 mm
10	70	28	average days: 36 when nos. 4 and 5 are ignored
12	90	41	
F1	67	36	
F3	95	60	Average temperature: 23° C
F4	71	26	Average CW: 77.6 mm
F5	88	55	Average days: 44.8
F6	55	38	Destalking month: November, 1966
F8	77	54	
F9	87	44	
F10	80	47	
F11	72	53	
F12	74	35	
F17	94	51	
F19	75	33	Average temperature: 22° C
F23	82	43	Average CW: 77.5 mm
F24	88	82	Average days: 45.7
F26	59	28	Destalking month: March, 1967
F29	67	37	

36 for 6 crabs of average CW 78 mm (or 32.4 days for 8 crabs of average CW 72 mm, when 2 considerably smaller crabs are included). The average temperature was 28° C. In the group destalked in November, the average number of days was 44.8 for 10 crabs with average CW 77.6 mm. The average temperature was 23° C. In the group destalked in March, there were 6 crabs that responded by molting. Their average CW was 77.5 mm and the average number of days was 45.7. The average temperature was 22° C.

The salinity during the period of the above 3 experiments varied between 32–34 ‰ which was slight. Assuming that the morphogenetic effect of light was negligible after destalking, the only factor left for comparison among these three experiments was temperature, since salinity changes were not significant, and the same food was used in each experiment. The average size of the first group (when only the largest 6 crabs were considered) was very close to those of the second and third group.

The results indicated that the fastest response to destalking occurred at the highest temperature within the tested range and generally decreased as the temperature fell. In spite of the slightly larger size in the first group, the crabs molted faster. These data clarify the relationship between temperature and growth, the optimal conditions falling in the same month as ovarian development.

From this work, both molting and spawning should have their optima occurring in the warmest month. This month could become the peak of spawning but not molting, only if molting is inhibited by reproduction. Once this was established, the difference between the patterns of the histograms of molting and spawning in Figure 2 could easily be explained.

Jegla (1965) found in the cave crayfish *Orconectes pellucidus inermis,* living in nature under almost uniform temperature conditions throughout the year, that eyestalk removal was followed by molting more slowly in autumn than in summer. He suggested that an ecdysis had occurred in his crayfish in nature not long before they were collected for his autumn experiment. His reason why the autumn batch took longer to reach the subsequent ecdysis was thus attributed to their destalking earlier in the molt cycle than the same operation on the summer batch.

The present work shows that the number of days between destalking and molting increased from the first to the third experiment (Table IX), *i.e.,* from summer to winter, yet, the third experiment included un-molted control crabs left over from the second, and the second, those from the first. Obviously, no ecdysis had occurred in these crabs between experiments. Thus, it appeared to be the lowering of experimental temperatures, and not destalking earlier in the molt cycle, that lengthened the periods required for the crabs to molt.

Endocrine functions at post-molt

Second event after molting in destalked crabs In order to observe the second event (molting or spawning) after molting, destalked crabs were maintained in the laboratory as long as they survived. Results are given in Table X, which only includes crabs responding to the operation by molting. Crabs that died within a few days after molting were also excluded since their death was apparently not related to a second event. From the Table, crabs that were destalked in February and March and that responded by molting all spawned subsequently. Those

TABLE X

Second event after molting in destalked adult female stone carbs

No.	CW	Destalked	Molting	Second event	Date of event
4	54–72	Aug. 31, 1966	Sep. 19, 1966	Spawned few eggs	Oct. 11, 1966
5	53–73	Aug. 31, 1966	Sep. 25, 1966	Spawned few eggs	Oct. 18, 1966
8	72–94	Aug. 31, 1966	Sep. 23, 1966	Spawned few eggs	Oct. 18, 1966; Jan. 3 and Feb. 28, 1967
F4	71–90	Nov. 2, 1966	Nov. 28, 1966	Spawned few eggs	Jan. 18, 1967
F9	87–103	Nov. 2, 1966	Dec. 16, 1966	Spawned few eggs	Feb. 20; Apr. 11; May 20, 1967.
				Dead	May 21, 1967
F10	80–100	Nov. 2, 1966	Dec. 19, 1966	Spawned few eggs	Feb. 18; Apr. 9, 1967
				Dead	Apr. 30, 1967.
F11	72–92	Nov. 2, 1966	Dec. 25, 1966	Spawned few eggs	Mar. 28, 1967
				Dead with brittle shell	Apr. 30, 1967
F14	95–110	Nov. 2, 1966	Dec. 12, 1966	Spawned few eggs	Feb. 24, 1967
				Dead	Mar. 8, 1967
F1	67–84	Nov. 2, 1966	Dec. 8, 1966	Dead, ovary white	Feb. 3, 1967
F3	95–109	Nov. 2, 1966	Jan. 1, 1967	Dead, ovary resorbed	Jan. 25, 1967
F5	88–106	Nov. 2, 1966	Dec. 27, 1967	Dead, ovary resorbed	Mar. 10, 1967
F6	55–73	Nov. 2, 1966	Dec. 10, 1967	Dead, ovary undeveloped	Mar. 2, 1967
F7	85–109	Nov. 2, 1966	Nov. 28, 1967	Dead, with few mature eggs in oviduct	Apr. 14, 1967
F12	74–92	Nov. 2, 1966	Dec. 5, 1966	Dead, ovary resorbed shell brittle	Apr. 30, 1967
C	74–93	Feb. 8, 1967	Mar. 20, 1967	Spawned	Apr. 27, 1967
				Dead, ovary medium in size.	Jul. 7, 1967
10	86.5–104	Feb. 7, 1967	Mar. 30, 1967	Spawned	Apr. 30, 1967 Jun. 5, 1967 Aug. 22, 1967
F17	94–?	Mar. 24, 1967	May 14, 1967	Spawned	Jun. 16, 1967
				Dead	Jul. 17, 1967
F23	82–103	Mar. 24, 1967	May 6, 1967	Spawned	Jul. 27, 1967
				Dead	Jul. 28, 1967
F29	67–84	Mar. 24, 1967	Apr. 30, 1967	Spawned	May 28, 1967; Jul. 9, 1967 Jul. 28, 1967.

destalked in August or November either spawned or died subsequent to molting. Further, those that spawned produced fewer eggs than those operated in the spring. Among those that died, one contained a small number of mature eggs in the oviducal region, which could only happen if the crab had recently spawned. All the rest did not have a mature ovary.

The results suggest that while the crabs included in the Table molted following destalking, the operation also caused spawning after molting, even within the off-season when ovarian development rarely occurred.

Destalking of post-molt adult female crabs As post molt crabs were not easily available, only a few were employed in this experiment. In Table XI, 4 crabs were destalked 2 to 6 days after they had molted in the laboratory, while 2 others were operated on a little over one month after molting. All operated crabs responded by spawning, although they were destalked in the non-spawning season. Crab number 24 spawned only a small number of eggs because it was destalked in a period during which little egg development had taken place.

Conclusions In these experiments, both molted destalked crabs and destalked postmolt crabs spawned even in an environment under which egg development and spawning rarely took place (compare with Table III and IV for intermolt crabs). Although destalked crabs at postmolt did not appear to accelerate ovarian development, it is possible that crabs early in their molt cycle tended to spawn, while those reaching premolt stage showed a tendency to molt. This is not difficult to explain since newly molted crabs had probably passed their highest titer of molting hormone. At intermolt, the tendency depends on the ovarian development which in turn depends on seasonal factors (above).

Finally, it is interesting to note that spawning could take place in such crabs without a normal amount of mature eggs during off-season. This finding deserves further investigation, as it may be reflected by the existence in the eyestalks, of separate controlling mechanisms for spawning and egg development.

Table XI

Destalking experiment on adult female post-molt stone crabs in winter-spring

Crab no.	CW	Date molted	Destalked	Spawned	Post spawned molting	Dead
24	81	Oct. 26, 1966	Nov. 2, 1966	Dec. 27, 1966	—	Jan. 22, 1967
F30	62	Jan. 8, 1967	Jan. 11, 1967	Feb. 22, 1967	Apr. 23, 1967	Aug. 9, 1967
F31	100	Jan. 10, 1967	Jan. 13, 1967	Mar. 6, 1967	—	Apr. 30, 1967
F32	103.7	Dec. 4, 1966	Jan. 18, 1967	Mar. 7, 1967	—	
F33	81	Mar. 23, 1967	Mar. 25, 1967	Apr. 20, 1967	—	Apr. 30, 1967
23	115	Jan. 2, 1967	Feb. 7: 1967	Mar. 14, 1967	—	

Discussion

Based on this work the adult female phase of the life cycle of *M. mercenaria* could be described as follows:

After the crab reaches maturity, the development of its ovary is probably initiated in nature during seasons of increased light intensity and warmer temperatures. Subsequent development seems favored by warm temperature alone. This may be the reason why the spawning peak did not coincide with the day-length peak. Because of the egg development, growth is inhibited, in spite of the fact that warmer temperatures also favor growth. After each spawning, the incubation of the eggs carried under the abdomen also inhibits molting. The mechanisms by which incubation inhibits molting are still unknown. The shift of season begins probably as the temperature drops in autumn. By this time new egg formation could be arrested, probably through diminishing light intensity. As soon as the latest batches are mature, spawned, and hatched after incubation in high summer, a new molting season begins in the fall. Even during the spawning season, an ecdysis could occur between two spawnings, whenever there is a lack of molt inhibition. It is likely that growth is possible all year around even in the coldest month, since the temperature in this tropical area rarely drops below the limit of molting blockage (Passano, 1960b). (The lowest monthly average from Figure 1 was slightly below 20° C, whereas from unpublished data on culturing this species, about 15° C has been found to be within the range of the blockage.) This would result in the molting season being distorted towards autumn when the temperature is less favorable. While reproduction and growth are antagonistic to each other, it is reproduction that inhibits molting; there is no evidence in any time of the year that molting could inhibit reproduction. In the pre-adult stages, *i.e.* juvenile and larval, when growth is the predominant activity, growth should proceed maximally in summer when the temperature is highest.

The alternation of dominance between molting and spawning is controlled by external factors as well as the molting cycle. While previous work on other species (Drach, 1944) demonstrated that once premolt was initiated the cycle proceeded to the molt, the present study suggests that at postmolt, spawning is dominant in adult female *Menippe,* irrespective of seasons. However, spawning and ovarian development are probably under separate control. During the intermolt stage, dominance depends also on external factors. Crabs within the intermolt stage could molt following destalking at one time, whereas, they could spawn if the operation is performed later in a different season. Obviously, this is determined by the state of ovarian development. Thus, along the change from postmolt via a long intermolt to premolt, crabs do not necessarily transfer irreversibly from the dominance of spawning to that of molting since, during the intermolt stage, the dominance of molting may be changed back to that of spawning if the ovaries mature.

As suggested in the above, at the spawning season, the process of ovarian development initially, at least, inhibits somatic growth. Later, after each spawning, the incubation process inhibits molting.

When one considers the well known effects of the endocrine system in a crab, it is possible that molting could be inhibited in one or more of the following ways: (a) an increase in circulatng MIH, (b) a decrease in concentration of OIH, (c) an increase in the effective concentration of ovarian promoting hormone from certain neurosecretory cells in the CNS, (Otsu, 1963) or (d) a decrease in secretion of molting hormone by the Y-organs (Démeusy, 1962).

If the regulation is entirely effected by (a) and (b) it is easy to imagine that any visual stimulus affecting MIH or OIH secretion would act through the eyes, as there should be some adaptive reason for the X-organs to be situated so near the eyes. This is most likely the case only when light intensity is concerned, but certainly not temperature, which acts through the whole organism. Also, what stimulates the MIH activity if the inhibition of molting is due to egg-bearing? If the egg-bearing pleopods receive their stimuli by way of the nervous system, would the stimuli not reach the thoracic ganglion and brain before they finally reach the X-organ? In this case, would it not be possible as well as simpler if inhibition could be effected by method (c)? If this is the case, a forward step is made towards the understanding of this problem since Scudamore (1948). It is not surprising that all the above four suggested processes could happen naturally. So far, the relation between the X-organ and Y-organ seems to be well established, that between the X-organ and the ovarian accelerating neurosecretory activities in the

CNS may also exist. A possible antagonistic relation between the Y-organ and the ovarian accelerating hormone-producing neurosecretory cells has not yet been investigated.

The estimate in the above that there were 4.5 spawnings to a molt in the average adult female crab has not taken into consideration the fact that the crabs were isolated. No male crabs were introduced for copulation at each molting. Although it has been proved (Cheung, 1968) that viable sperm could be retained after molting in the female stone crab, it is still uncertain whether a copulation with introduction of new sperm into the female would have any effect on ovarian development as well as on the frequency of spawning. This should certainly deserve future investigation.

Finally, I like to compare the biology of *M. mercenaria* with that of another crab, *Carcinus maenas* which can also molt after reaching maturity. In the latter species inhabiting waters in Western Europe, growth as well as egg development is blocked by low temperatures (Démeusy, 1964), so that the molting season is predominantly in summer when the temperature regimen is most favorable.

Destalking adult *Carcinus* has been found to cause only ovarian growth and not molting in any season (Cheung 1964, Démeusy 1965b). It was during the juvenile stage and the terminal anecdysis (Carlisle 1957) that it would respond to the operation by molting. These may not be specific differences only between *Carcinus* and *Menippe*; they may also distinguish similar crabs distributed over colder and warmer waters, respectively.

I am grateful to Dr. C. E. Lane for useful help and suggestions in the preparation of this manuscript. I thank Drs. A. Provenzano, Jr. and H. Moore for access to their temperature data for comparison. I am indebted to Mr. James Cox for technical assistance, and Mr. A. Mendez for the supply of local environmental data.

Summary

1. The effects of certain environmental and physiological factors in the growth and reproduction of adult female stone crabs, *Menippe mercenaria* (Say), were studied between April, 1965 and November, 1967 by employing a long-term rearing method. The crabs were kept isolated from one another in tanks supplied with constantly running sea water pumped almost directly from Biscayne Bay and were fed daily with pink shrimp meat.

2. Results showed that spawning was most frequent in warm temperature, indicating that the seasonal dependence of spawning may be related to seasonal temperatures. Molting occurred most frequently in autumn-winter, a period of decreased spawning, although summer temperature seemed more favorable to molting. Summer molting may therefore be inhibited by reproductive activities.

3. In order to study the control of molting and spawning, several experiments involving the destalking of crabs were performed in a 13-month period. Destalking in August and September of 1966 induced molting. After September, crabs responded to destalking either by molting or by spawning. As the year progressed, the proportion of spawning to molting crabs increased until in September, 1967

spawning was the only response. This finding not only supported the work on *Gecarcinus lateralis* (Weitzman, 1964) in that there was a cyclic change in the dominance of molting and spawning, but also indicated a transitional period between the periods when molting or spawning are dominant.

4. The effect of destalking postmolt crabs was studied. The results indicate these crabs spawn precociously, but do not undergo accelerated ovarian development. This indicates that spawning and ovarian development may be controlled by different hormones.

LITERATURE CITED

Bauchau, A. G., 1961. Regeneration des pereiopodes et croissance chez les Crustaces decapodes Brachyoures. 1.—Conditions normales et Role des Pedoncules oculaires. *Ann. Soc. Roy. Zool. Belgique.*, **91** (1) : 57–84.

Bliss, D. E., 1953. Endocrine control of metabolism in the land crab, *Gecarcinus lateralis* (Freminville). 1. Differences in the respiratory metabolism of sinus glandless and eyestalkness crabs. *Biol. Bull.*, **104** : 275–296.

Bliss, D. E., and J. R. Boyer, 1964. Environmental regulation of growth in the decapod crustacean *Gecarcinus lateralis*. *Gen. Comp. Endocrinol.*, **4** (1) : 15–41.

Brown, F. A. Jr., and G. M. Jones, 1949. Ovarian inhibition by a sinus-gland principle in the fiddler crab. *Biol. Bull.*, **96** : 228–232.

Carlisle, D. B., 1957. On the hormonal inhibition of moulting in decapod Crustacea. II. The terminal anecdysis in crabs. *J. Mar. Biol. Ass. U.K.*, **36** : 291–307.

Carlisle, D. B., and F. Knowles, 1959. *Endocrine Control in Crustaceans.* Cambridge University Press, 120 pp.

Cheung, T. S., 1964. Aspects of reproduction of the female shore crab, *Carcinus maenas* (L) and some related decapods. *Ph.D. thesis, University of Glasgow.*

Cheung, T. S., 1966. The inter-relations among three hormonal-control characters in the adult female shore crab, *Carcinus maenas* (L). *Biol. Bull.*, **130** : 59–66.

Cheung, T. S., 1968. Transmolt retention of sperm in the adult female stone crab, *Menippe mercenaria* (Say). *Crustaceana*, **15** : 117–120.

DeLeersnyder, M., 1967. Influence de la salinite et de la ablation des pedoncules oculaires sur la mue et sur le development ovarien d' *Eriocheir sinensis* H. Milne-Edwards. *Cah. Biol. Mar.*, **8** : 421–435.

Démeusy, N., 1962. Observations sur la maturation ovarienne du Crabe *Carcinus maenas* (L) apres ablation des glandes de mue chez les femelles adults. Premiers resultats. *C. R. Seances Acad. Sci. Paris*, **255** : 3062–3064.

Démeusy, N., 1963. Rapports entre mue et vitellogenese chez le crabe *Carcinus maenas* (L). *Proc. XVI Int. Congr. Zool.*, **2** : 118.

Démeusy, N., 1964. Influence de divers facteurs sur la croissance somatigue et la vitellogenese du Crabe *Carcinus maenas* (L). *C. R. Acad. Sci. Paris.*, **258** : 5992–5994.

Démeusy, N., 1965a. Croissance somatique et fonction de reproduction chez la femelle du decapode brachyoure *Carcius maenas* Linne. *Arch. Zool. Exp. Gen.*, **106** : 625–644.

Démeusy, N., 1965b. Bouveaux resultats concernant les relations entre la croissance somatique et la fonction de reproduction du Decapode Brachyoure *Carcinus maenas* L. Cas des femelles de printemps. *C. R. Acad. Sci. Paris*, **260** : 2925–2928.

Démeusy, N., 1965c. Nouveaus resultats concernant les relations entre la croissance somatique et la fonction de reproduction du Decapode Brachyoure *Carcinus maenas* L. Cas des femelles survies pendant l'hiver. *C. R. Acad. Sci. Paris*, **260** : 323–326.

Drach, P., 1944. Etude preliminaire sur le cycle d'intermue et son conditionnement hormonal chez *Leander serratus* (Pennant). *Bull Biol. France Belgium*, **78** : 40–62.

Jegla, T. C., 1965. Studies of the eyestalks of cave crayfish. *Amer. Zool.* **5** (4) : 94.

Noe, C. D., 1967. Contribution to the life history of the stone crab, *Menippe mercenaria* (Say), with emphasis on the reproductive cycle. *M. S. thesis, University of Miami*, 55 pp.

Otsu, T., 1963. Bihormonal control of sexual cycle in the freshwater crab, *Potamon dehaani*. *Embryologia*, **8** : 1–20.

Passano, L. M., 1953. Neurosecretory control of molting in crabs by the X-organ sinus gland complex. *Physiologia Comp. Oecol.*, **3**: 155–189.

Passano, L. M., 1960a. Molting and its control, 473–536. *In*: *Physiology of Crustacea, Vol. 1.* Academic Press, New York.

Passano, L. M., 1960b. Low temperature blockage of molting in *Uca pugnax*. *Biol. Bull.*, **118** (1): 129–136.

Scudamore, H. H., 1948. Factors influencing molting and the sexual cycles in the crayfish. *Biol. Bull.*, **95**: 229–237.

Truitt, R. V., 1939. Our water resources and their conservation. *Chesapeake Biological Laboratory, Contribution* **27**: 10–38.

Weitzman, M. C., 1964. Ovarian development and molting in the tropical land crab, *Gecarcinus lateralis* (Freminville). *Amer. Zool.* **4**: 329–330.